Extractables Guide

LeCouple® LSC Sterile Connectors

Shanghai LePure Biotech Co., Ltd

Catalogue

1. Introductions

The pharmaceutical single use system produced by Shanghai LePure is widely used in the bio-pharmaceutical process. Its main application fields include the research, development and production of antibody, vaccine and cell therapy products. At present, pharmaceutical single use systems are widely used in upstream, downstream and final filling processes. Therefore, end users must fully understand and verify their interactions with bio-pharmaceutical solutions and final drug products. In order to ensure the product quality, the pharmaceutical enterprises shall conduct comprehensive analysis and testing in the early stage of process development and within the scope of process monitoring and quality control, so as to prove the purity, efficacy and safety of drugs. Product safety assessment shall be carried out in the process of design and development of the single use system. These validation studies involve complete quantitative, identification and toxicological assessment of the leachables, which are the substance remained in the drug solution due to the interaction between the drug solution and the pharmaceutical single use system. Leachables are a subset of the extractables that can be extracted from pharmaceutical single use systems. Usually, the solvents and conditions for extractables testing are more severe than that for leachables. The purpose of this Guide is to provide the worst-case data on extractables to support the validation studies conducted by process developers and toxicologists.

The safety problem of disposable components, which is the most common problem in the pharmaceutical single use system, has always been the most concerned problem of many pharmaceutical enterprises, especially for the safety of extractables. For this kind of disposable components, LePure has referenced the

technical data of foreign raw material suppliers, the technical guidelines for compatibility studies published by domestic CDE (including Technical Guidelines for Compatibility Studies of Chemical Injection and Pharmaceutical Glass Packaging Container (Tentative), Technical Guidelines for Compatibility Studies of Chemical Injection and Plastic Packaging Material (Tentative), and Technical Guidelines for Compatibility Studies of Chemical Injection and Elastomeric Seals (Tentative) in China; and the Application and Technical Guidelines for Single Use System (Tentative) published by the China Center for Food and Drug International Exchange in November 2017), the technical guidelines formulated by relevant SUS organizations, and USP <665> and USP <1665>and developed a reasonable test scheme for the dissolved substances in single use system.

The potential dissolved substances in the pharmaceutical single use system produced may come from surfactants, lubricants and additives in the process of plastic processing, or from the shedding of raw materials of material structure and oligomer monomer. This study report summarizes the information on extractables from disposable components in which includes elements and organic compounds (nonvolatile compounds, semi-volatile and volatile compounds, small-molecule volatiles) in three kinds of simulated solvents, mainly referring to USP<665>, BPOG guide for pharmaceutical single use system. Elements were detected by inductively coupled plasma-mass spectrometry (ICP-MS), non-volatile compounds were detected by high performance liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), semi-volatile and volatile compounds were detected by gas chromatography-mass spectrometry (GC-MS), and small-molecule volatiles were detected by headspace gas chromatography-mass spectrometry (HS-GC-MS).

2. Purpose and Methodology of Extractables Testing

According to the guidelines of USP < 665 > and USP < 1665 >, the extractables test scheme was formulated respectively, and the extractables were tested according to this scheme.

Before the test, LePure has confirmed the analysis and evaluation threshold (AET). According to the guidelines of Product Quality Research Institute (PQRI), AET was determined as a threshold. When the concentration of a compound exceeds the threshold, the compound should be identified, quantified and reported. In addition, the toxicity for this compound needs to be evaluated. AET is obtained through conversion according to the appropriate safety assessment threshold (SCT) or toxicological concern threshold (TTC), taking into consideration the dosage of the product. When the concentration of a compound is lower than the threshold, it can be considered that the toxicity of the compound is very low and will not be harmful to human. As LePure single use systems may be exposed to a variety of drugs and chemical reagents, and the maximum daily dosage of drugs cannot be confirmed at this stage, based on the minimum detection limit of the instrument, and according to the guide of BPOG, the limit of report was defined as 0.1μg/mL for organic compounds and 20ng/mL for inorganic substances for this study. Converted to the concentration for surface area, the limit of report was 0.030μg/cm^2 for organic compounds and 0.006μg/cm^2 for inorganic substances.

3. Information of Component and Instruments

3.1 Information of Component

Table 1 Information of Component

Name		Material	Part. No. for Testing
LeCouple® LSC Sterile Connectors	LeCouple® LSC 1/2"Hose Barb, male connector	PC	LSC1M01
	LeCouple® LSC 1/2"Hose Barb, female connector		LSC1F01

Note: This guide is applicable to other components constructed from the same materials.

3.2 Information of Instruments

Table 2 Information of Instruments

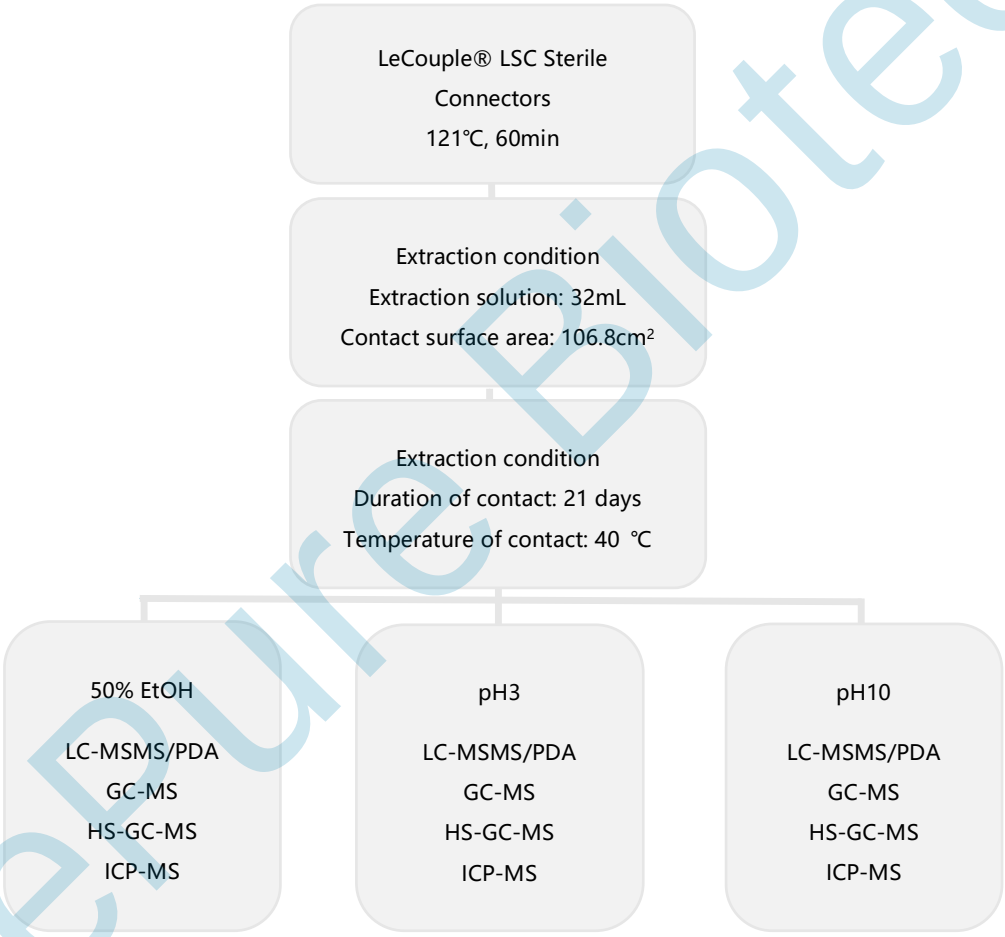
Name	Model/Specification	Manufacturer
Gas Chromatography-Mass Spectrometry	8890/5977B	Agilent
Headspace Gas Chromatography-Mass Spectrometry	7697A/8890/5977B	Agilent
High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry	Xevo TQ-S Micro	Waters
Inductively Coupled Plasma-Mass Spectrometry	7900	Agilent

4. USP < 665 > Compliance

4.1 Overview of Extractables Protocol

USP<665> guide was referred to determine the extraction solution, extraction method and test instrument used in extractable study. An overview of extractables study process is provided in Table 3.

Table 3 Extractable Study Process



4.2 Results of Extractables Testing

The elemental, non-volatile, semi-volatile and volatile extracts in LeCouple® LSC Sterile Connectors were detected. In addition, we focused on the antioxidants and their degradation products, fatty acids, phthalate plasticizers, polycyclic aromatic hydrocarbons, lubricants, siloxanes, vulcanizing agents, nitrosamines and other additives during the experiment and data analysis.

4.2.1 Results of Elemental Impurities

Table 4 Results of Elemental Impurities

Element	ICH Q3d Class	Conc. (μg/cm²)		
		50%EtOH	pH3	pH10
Cd	Class 1	ND	ND	ND
Pb	Class 1	ND	ND	ND
As	Class 1	ND	ND	ND
Hg	Class 1	ND	ND	ND
Co	Class 2A	ND	ND	ND
V	Class 2A	ND	ND	ND
Ni	Class 2A	ND	ND	ND
Tl	Class 2B	ND	ND	ND
Au	Class 2B	ND	ND	ND
Pd	Class 2B	ND	ND	ND
Ir	Class 2B	ND	ND	ND
Os	Class 2B	ND	ND	ND
Rh	Class 2B	ND	ND	ND
Ru	Class 2B	ND	ND	ND
Se	Class 2B	ND	< LOR	ND
Ag	Class 2B	ND	ND	ND
Pt	Class 2B	ND	ND	ND
Li	Class 3	ND	ND	ND
Sb	Class 3	ND	ND	ND
Ba	Class 3	ND	ND	ND
Mo	Class 3	ND	ND	ND
Cu	Class 3	ND	ND	ND
Sn	Class 3	ND	ND	ND
Cr	Class 3	ND	ND	ND
B	N/A	0.026	0.028	ND
Mg	N/A	< LOR	ND	ND

Element	ICH Q3d Class	Conc. (μg/cm²)		
		50%EtOH	pH3	pH10
Al	N/A	ND	ND	ND
Ca	N/A	ND	0.025	ND
Ti	N/A	ND	ND	ND
Mn	N/A	ND	ND	ND
Fe	N/A	ND	ND	ND
Zn	N/A	ND	ND	ND
Sr	N/A	ND	ND	ND
W	N/A	ND	ND	ND

Note: "LOR" means limit of report, and the concentration of LOR is 0.006μg/cm².

4.2.2 Results of Organic Compounds

Table 5 Results of Organic Compounds

Model Solvent	Analytical Method	Compound Name	CAS	Conc. (μg/cm²)
50% EtOH	HS-GC-MS	2-Methyl-2-propanol	75-65-0	0.183
		Trimethylethoxysilane	1825-62-3	0.050
		Octamethyltetrasiloxane	556-67-2	0.030
		1-((Trimethylsilyl)oxy)-1-(4-((trimethylsilyl)oxy)phenyl)propan-2-amine	N/A	0.055
	GC-MS	Methyl N-hydroxybenzenecarboximidoate	N/A	0.071
		Ethyl 4-ethoxybenzoate	23676-09-7	0.097
	LC-MS/MS	E-caprolactam	105-60-2	0.162
		2,6-Di-t-butyl-4-bromomethyl phenol	2091-51-2	0.100
		Unknown (SIR-, 223.0)	N/A	0.033
		2,6,10,14-Tetramethylhexadecane	638-36-8	0.120
		Unknown (PDA, 262nm)	N/A	0.022
		Unknown (PDA, 210nm)	N/A	0.109
		1,3:2,4-Bis (3,4-dimethylbenzylidene) sorbitol	135861-56-2	0.702
		Dioctyldiphenylamine	101-67-7	0.223
		Stearamide	124-26-5	0.428
		Ionox 220, Ethanox 702	118-82-1	0.075
		Bis(2,6-di-ter-butyl-4-methylphenyl) pentaerythritol-diphosphite	80693-00-1	0.195
		Unknown Siloxane (SIR+,573.2)	N/A	0.029
pH3	HS-GC-MS	Acetone	67-64-1	0.059
	LC-MS/MS	2,6,10,14-Tetramethylhexadecane	638-36-8	0.065
pH10	GC-MS	3,4'-Isopropylidenediphenol	46765-25-7	0.060
	LC-MS/MS	Unknown (PDA, 262nm)	N/A	0.079
		1,3:2,4-Bis (3,4-dimethylbenzylidene) sorbitol	135861-56-2	0.179

Note: "LOR" means limit of report. The concentration of LOR for LC-MS/MS is 0.015μg/cm², the concentration of LOR for GC-MS and HS-GC-MS is 0.030μg/cm².

5. Safety Assessment

The toxicity of extractables and leachables must be evaluated for the effects on both patients and process. Although almost any quantity of certain compounds in a drug is considered unacceptable (e.g., ICH Q3C class-1 solvents), the toxicity of extractables or leachables must be observed in the broader context of the following criteria, the actual concentration of the leachables in the final drug, the mode of administration, the dosage, the duration of treatment, the number of patients, and the risk benefit assessment.

Therefore, the toxicity is not only related to the identification and concentration of the extractables, or only related to the amount of leachables in the process fluid or pharmaceutical intermediates. The daily intake of patients can be obtained taking into consideration information of extractable concentration, model solvent volume, test component contact area, process component contact area, process batch and dosage. The daily intake of single compound can be compared with PDE value. For compounds or unknown substances whose PDE value cannot be obtained, the worst scenario can be assumed.

1) All extractables are migrating to the final product.
2) All extractables are considered as DNA reactive impurities (genotoxicant).

The purpose of determining the toxicological concern threshold (TTC) in ICH M7 is to define a common acceptable exposure level for compounds that have gone through toxicological studies (see Table 6). An appropriate limit value can be chosen based on treatment cycle and treatment route to conduct safety assessment.

Table 6 Acceptable Intakes for an Individual Impurity

Duration of treatment	≤1 month	>1-12 months	>1-10 years	>10 years to lifetime
Daily intake [μg/day]	120	20	10	1.5

Depending on the toxicity categorization and concentration of each detected compound and elements, there is no high-risk compounds or elements detected for LeCouple® LSC Sterile Connectors. The toxicological assessment of extractables or leachables for drug products should be performed based on the process conditions and clinical dose for patient.

Appendix 1: Study of Elements Impurity

Appendix Table 1 Instrument Method for Elements Impurity by ICP-MS

RF Power	1550W	**RF Matching**	1.8V
Peristaltic Pump Speed	0.10rps	**He Flow**	2mL/min
Sampling Depth	10mm	**Nebulizer Gas Flow Rate**	1.05L/min
Nebulizer Chamber Temperature		2°C	

Appendix 2: Study of Organic Compounds

Appendix Table 2 Scan Method for Semi-Volatile Compounds by GC-MS

Column	HP-5MS（30m×0.25mm, 0.25μm）		
Carrier Gas	He		
Flow Rate	1.2 mL/min		
Temperature	Injection Temp.: 260°C; Transferline Temp.: 300°C; MS Source Temp.: 230°C; Ms Quard Temp.:150°C.		
Acquisition Type	Full Scan 30-650; SIM+SCAN		
Temperature Program			
No.	**Rate (°C/min)**	**Temp. (°C)**	**Hold Time (min)**
1	/	60	1
2	10	170	3
3	15	300	8

Appendix Table 3 Scan Method for Volatile Compounds by HS-GC-MS

Column	DB-624 UI（60m×0.25mm, 1.4μm）		
Carrier Gas	He		
HS Oven Temp	80℃		
Manifold Temp	95℃		
Transfer Line Temp	110℃		
Vial Equilibration Time	30 min		
Flow Rate	12 mL/min		
Split Ratio	10:1		
Temperature	Injection Temp.: 250°C, Transferline Temp.: 230°C, MS Source Temp.: 230°C, Ms Quard Temp.:150°C.		
Acquisition Type	Full Scan: 35-320; SIM+SCAN		
Temperature Program			
No.	**Rate (°C/min)**	**Temp. (°C)**	**Hold Time (min)**
1	/	40	2
2	8	90	4
3	6	200	8

Appendix Table 4 Instrument Method for Other Non-volatile Compounds by LC-MSMS/PDA

Mass Condition			
Scan Type	SCAN+SIM+MRM	**Polarity**	Positive/Negative
Source Temp	150℃	**Cone Gas Flow**	50L/Hr
Desolvation Temp	500℃	**Desolvation Gas Flow**	800L/Hr
Liquid Chromatography Condition			
Column	ACQUITY UPLC®BEH C18 (50 mm*2.1 mm, 1.7 μm)	**Flow rate**	0.3mL/min
Column Temp	60℃	**Detector**	PDA
Scan Range	PDA: 230nm，210nm~400nm ESI±: 100-1200		
Elution Mode	Gradient Elution		
Mobile Phase	Phase A: MeOH (0.01% Formic Acid) Phase B: Water (0.01% Formic Acid)		
Time (min)	**A (%)**	**B (%)**	
0	10	90	
1	10	90	
2.5	20	80	
8	100	0	
10	100	0	
10.2	10	90	
12	10	90	

Reference

1. USP<665> Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products
2. USP<1665> Characterization and Qualification of Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products
3. BioPhorum Best Practices Guide for Extractables Testing of Polymeric Single-Use Components Used in Biopharmaceutical Manufacturing
4. BioPhorum Best Practices Guide for Evaluating Leachables Risk from Polymeric Single-Use Systems Used in Biopharmaceutical Manufacturing
5. ICH Q3D(R2) Guidelines for Elemental Impurities
6. ICH M7(R1) Evaluate and Control DNA Reactive (Mutagenic) Impurities in Drugs to Limit Potential Carcinogenic Risk
7. Application and Technical Guide of Disposable Use System

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